DIRECT ASSIMILATION OF ORGANIC CARBON BY CERATODON PURPUREUS[1]

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(WITH FIVE FIGURES)

Considerable attention has been devoted in recent years to the investigation of the assimilation by green plants of carbon in organic form. Attention has been directed to this phase of plant physiology because of the renewed interest in the relation of the organic compounds found in the organic material of the soil to the growth of green plants, and also because of the light which the result may throw on the question of the products formed in photosynthesis and of the function of various organic compounds in plant metabolism. A number of investigators have shown that higher plants may absorb and assimilate many organic compounds. In 1914 the writer began an investigation of the assimilation of organic compounds by the mosses. Circumstances made it impossible to complete the investigation. The results, however, show some facts and may prove suggestive to those who may continue the work.

SERVETTAZ (6) and VON UBISCH (7) have made observations upon the assimilation of organic carbon by the mosses. SERVETTAZ grew several species of mosses under sterile conditions on various solid and liquid media. Most of his work was done with *Hypnum purum*. According to SERVETTAZ the mosses when furnished with sugar or some other organic substance are able to live in the dark and become green slowly; but under these conditions they do not form starch and their increase is never important. Levulose, lactose, maltose, and saccharose when present at a concentration of 5 parts per 1000 favor development, but 2 parts per 100 are decidedly toxic. Dextrine, starch, and gum arabic at a concentration of 5 parts per 1000 retard development, but at 2 parts per 1000 favor it. *Hypnum purum* prefers the

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hexoses. Grown in the light in a mineral solution containing sugar, SERVETTAZ found that in 4 months *Hypnum purum* assimilated sugar, as given in table I.

TABLE I

Sugar	Original amount of sugar	Sugar used
Glucose	0.25 gm.	0.07 gm.
Levulose	0.25	0.065
Lactose	0.25	0.01
Maltose	0.25	0.005
Cane sugar	0.25	0.012

SERVETTAZ also found that peptone is assimilated by the mosses if present in concentrations below 2 parts per 1000. Inulin apparently is not assimilated.

The observations by VON UBISCH on the assimilation of organic carbon by the mosses were few. He grew several species of mosses in pure culture and noted the presence of large starch grains in the protonema of *Funaria hygrometrica* grown in the dark on a nutrient agar containing peptone and glucose. On the same agar lacking peptone and glucose the starch grains were very small.

Investigation

Although SERVETTAZ and VON UBISCH both obtained pure cultures from the spores in the capsules of various mosses, the moss used in this work was accidentally obtained in pure culture. It was found growing as a contamination in one of the culture vessels used by KNUDSON (3) in his investigation of the assimilation of organic compounds by the higher plants. Transferred to a nutrient agar it grew well. The protonema penetrated the soft agar and moss plants eventually were produced. It was identified as *Ceratodon purpureus* L. by Dr. A. L. ANDREWS of Cornell University, to whom the writer expresses his thanks.

USE OF ORGANIC CARBON.—Preliminary experiments showed that *Ceratodon purpureus* can assimilate organic carbon. In test tubes on a nutrient agar containing glucose the growth in the light was 4 or 5 times as luxuriant as on the nutrient agar lacking glucose. The heavy dark green mat formed on the glucose agar is shown in fig. 1. This photograph was made 1 month after inoculation.

In solution cultures the utilization of the glucose was shown even more clearly. Fifty cc. of Czapek's nutrient solution for fungi (2) plus 0.1 gm. of calcium chloride per liter was placed in 125 cc. Erlenmeyer flasks. To some of the flasks 3 per cent glucose was added. All were sterilized and inoculated with the

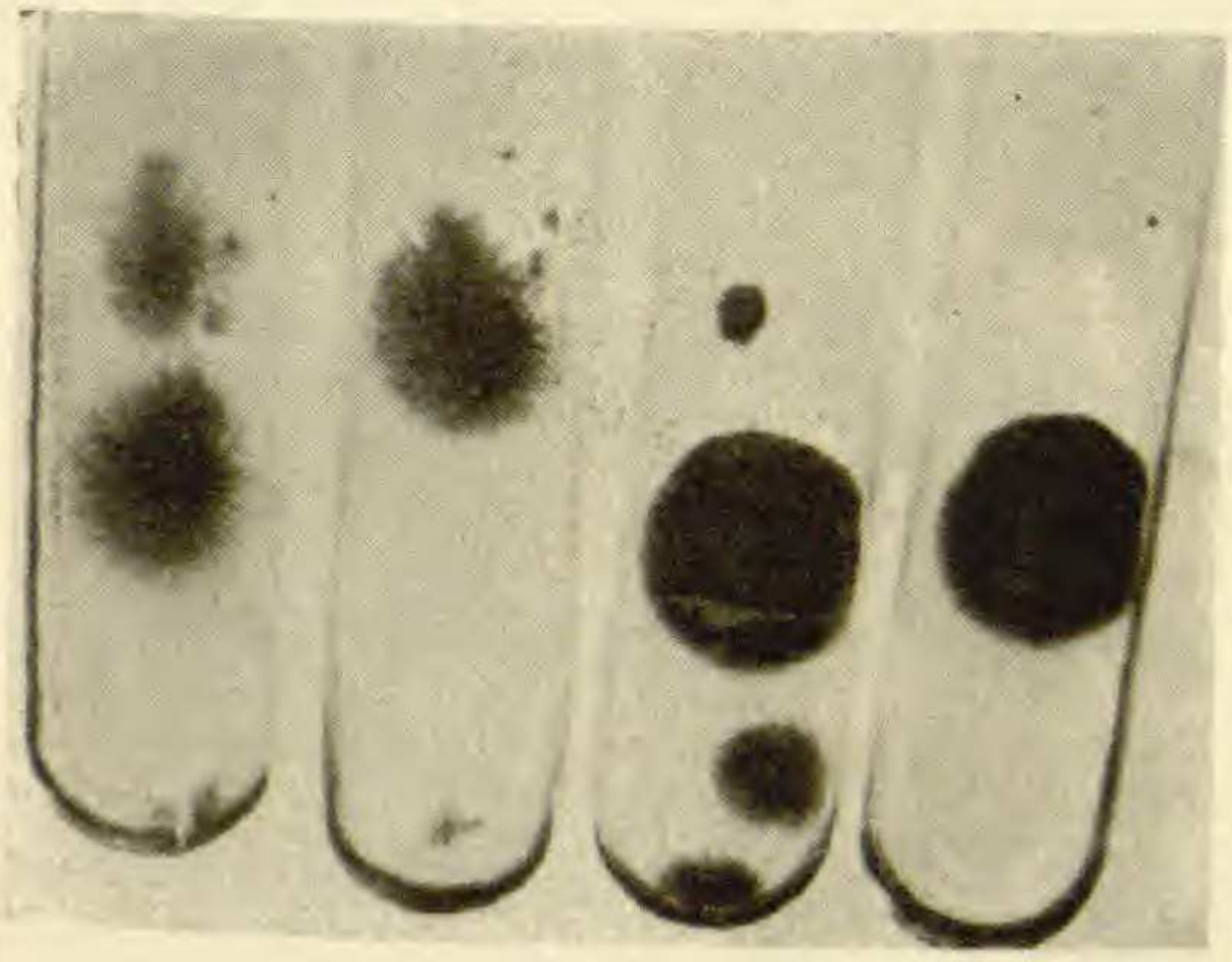

FIG. 1

FIG. 2

FIGS. 1, 2.—Fig. 1, *Ceratodon purpureus* grown 1 month in light on nutrient agar: 2 tubes to left contain no glucose; 2 tubes to right contain glucose; fig. 2, *Ceratodon purpureus* grown in dark for 1 month in modified Czapek's solution: flask to left contains 3 per cent glucose; flask to right contains no organic compound.

moss by transferring a bit of the protonema growing on agar in a test tube. Some of the flasks were placed in a north window and others in a dark cupboard. At the end of a month and a half it was found that in the light far more growth had occurred in the flasks containing glucose. In the glucose solution only protonema had developed; in the check young moss plants had been formed. In the dark no growth had occurred in the check, while the solutions in those flasks containing glucose were completely filled with a mass of dark reddish brown colored protonema (fig. 2).

AVAILABILITY OF DIFFERENT FORMS OF CARBON.—The culture solution used was one devised by MOORE for the culture of algae, and is described by REED (5). The carbon compounds were all Merck's products. Dextrose, pure, "Mulford" and Schering's levulose were also used in repeating some of the experiments.

Sufficient of the organic compound was added to make a concentration of 0.1 mol. The culture vessels were 125 cc. Erlenmeyer flasks, containing 50 cc. of solution. Those sugars, such as cane sugar, which could be hydrolyzed were sterilized in an Arnold sterilizer and tested for hydrolysis before use. After sterilization

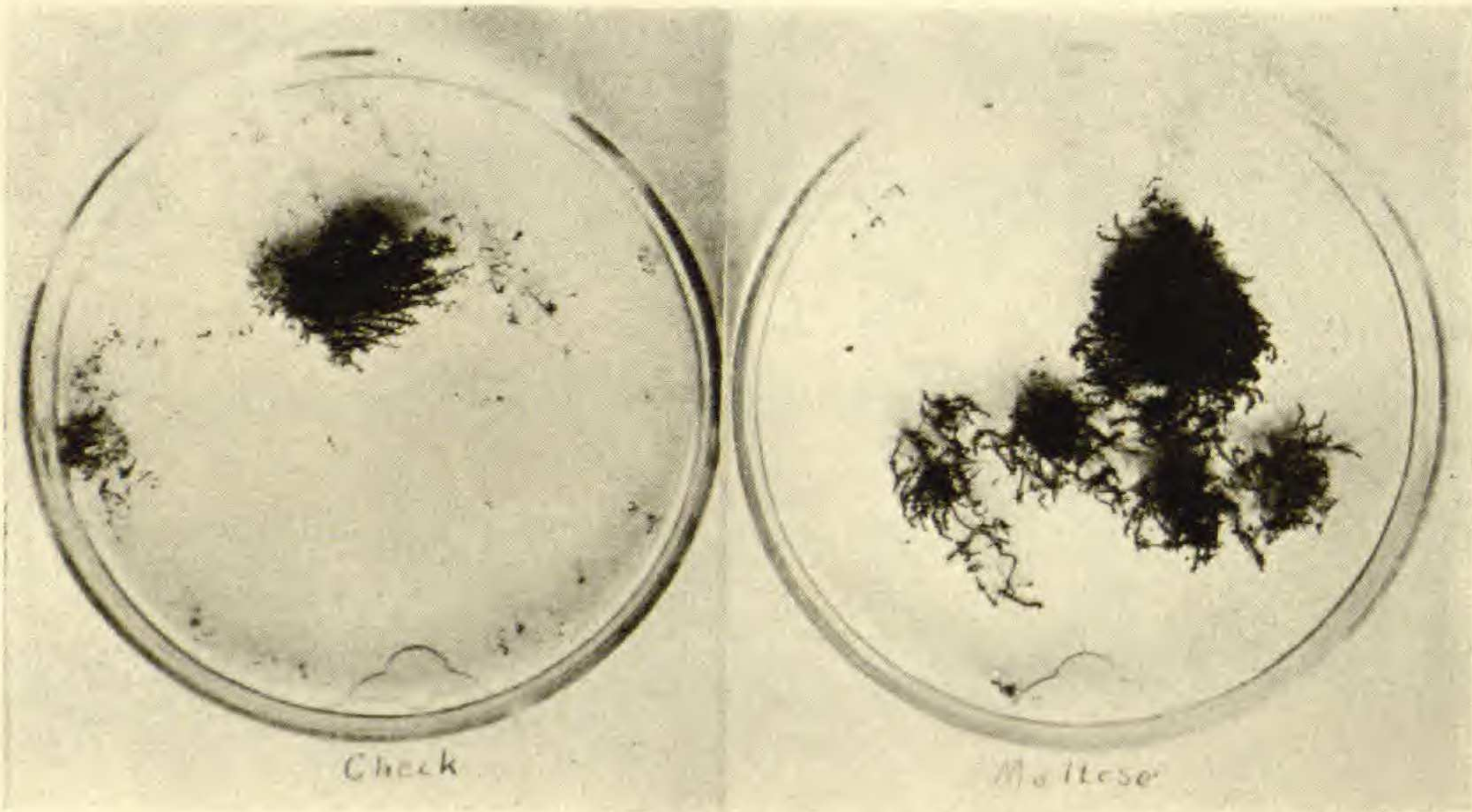

FIG. 3.—*Ceratodon purpureus* grown in nutrient solution in light for 2.5 months: on left grown without organic compound; on right grown in 0.1 mol. maltose.

the flasks were inoculated, as previously described, with the moss protonema. The moss was grown for 2.5 months in the presence of each carbon source in triplicate culture both in the light and in the dark.

In the dark the moss grew in the levulose, glucose, cane sugar, maltose, galactose, and lactose solutions. The amount of growth was greatest with levulose as the source of carbon. In the galactose and lactose solutions the growth was very slight. No growth, save a slight lengthening of the filaments of the original material, occurred in the check, nor in the presence of mannite, glycerine, or starch. In all cases in the dark the growth consisted of protonema. No moss plants were produced. The protonema, instead of having the familiar yellow color of chlorotic higher plants, was a dark reddish brown.

By the use of iodine starch was demonstrated in the protonema grown in the levulose, glucose, cane sugar, maltose, galactose, and lactose solutions. The protonema grown in the levulose solution contained the most starch. At the end of the experiment it was found that the cane sugar was completely inverted. The use of Barfoed's solution and the osazone test failed to demonstrate the

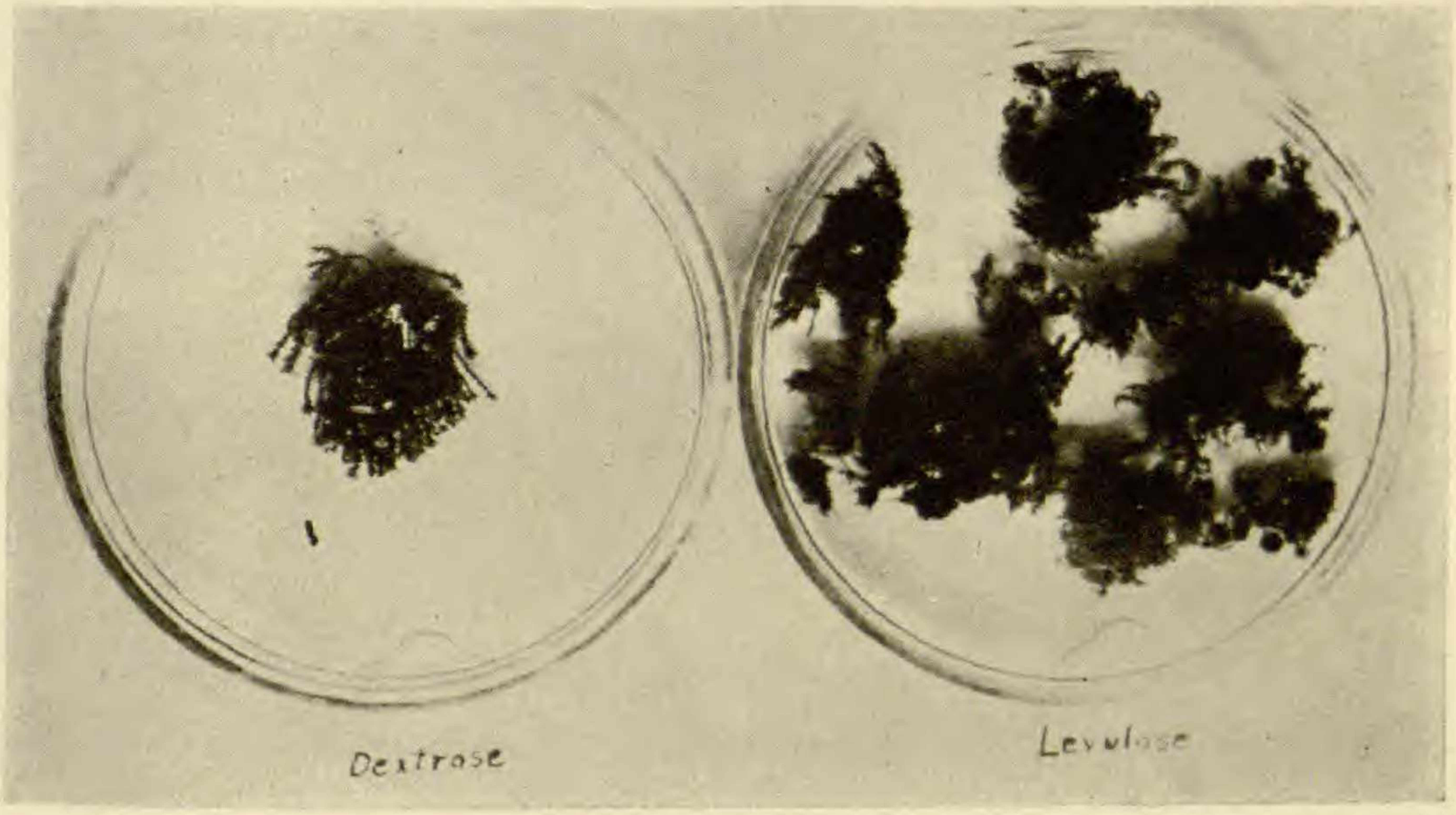

FIG. 4.—*Ceratodon purpureus* grown in nutrient solution in light for 2.5 months: on left grown in 0.1 mol. dextrose; on right in 0.1 mol. levulose.

presence of glucose in the maltose solution. No glucose was found in the lactose solution.

In the light there was growth in all the cultures, showing that none was toxic to the moss. The greatest amount of growth was found in the levulose solution. Moss plants developed in all cultures. To some extent the macroscopic appearance of the growth in the light seemed to be influenced by the particular sugar used. For example, in the glucose solution sharp clean cut moss plants were produced. In the levulose solution many moss plants were formed, but they were shorter and thicker. This difference in the moss plants and the excess of protonema in the levulose solution gave the culture as a whole a woolly appearance. The effects of glucose, levulose, and maltose on the moss are shown in figs. 3 and 4. The cultures also differed in color. The protonema in the levulose, glucose, and cane sugar was brownish at the end of the experiment; while in the lactose, maltose, and check it was still a normal green.

COMPARISON OF LEVULOSE AND GLUCOSE AS CARBON SOURCES.—In the preceding experiment the growth when levulose was the source of carbon was so much greater in amount (fig. 5) than that when glucose was the source of carbon that a further comparison of the effects of the two sugars was made. The moss was grown from

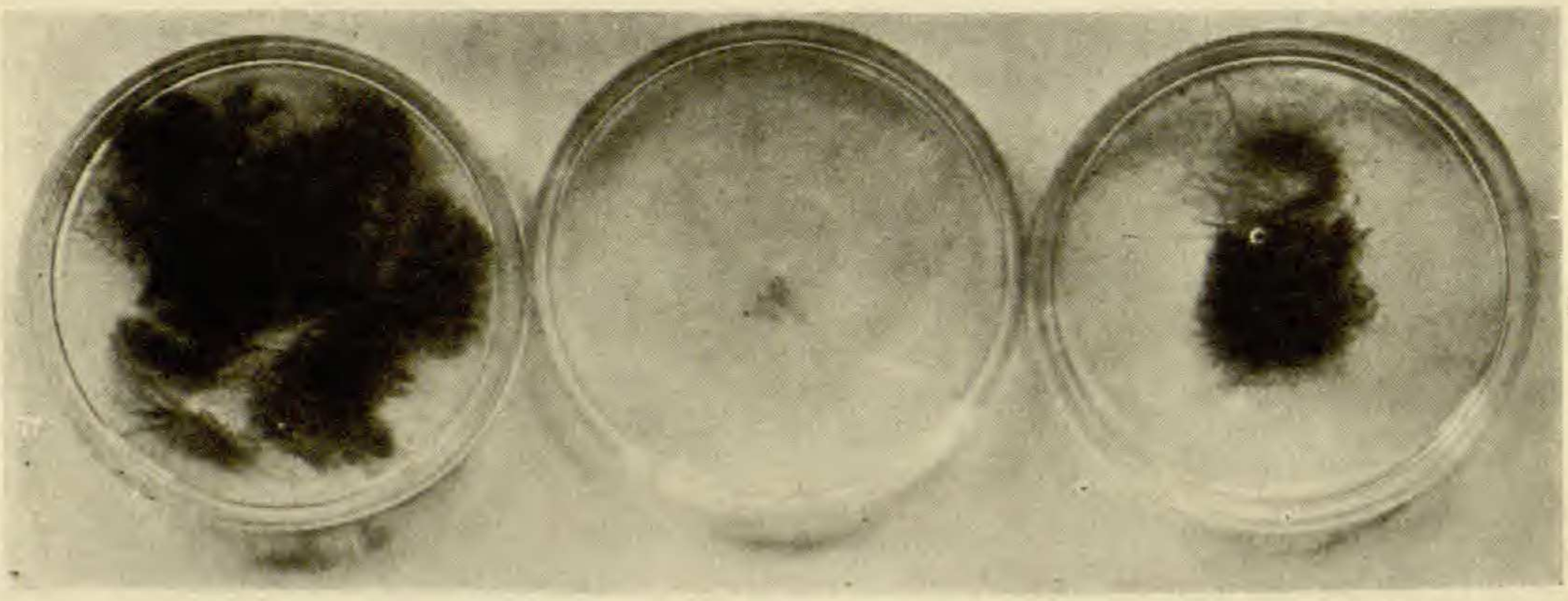

FIG. 5.—*Ceratodon purpureus* grown in dark for 2 months in nutrient solution: from left to right, 0.1 mol. levulose, no carbohydrate, 0.1 mol. glucose.

November 27 to February 24 in the modified Czapek's solution mentioned. Triplicate cultures were grown in the light and in the dark. The dry weight of the moss was determined by filtering the protonema and moss plants into a Gooch crucible and drying at 110° C. The sugar determinations were made by the use of Fehling's solution. The results are given in table II and represent the averages of the data for triplicate cultures.

The sugar analyses given in table II show an unmistakable consumption of sugar in all cases. More levulose was used than glucose. In the case of levulose the greater consumption of sugar occurred in the dark. In the case of glucose the greater consumption occurred in the light. Comparing the dry weights of the moss protonema and plants in the check, glucose, and levulose solutions, it is evident that the sugar has greatly increased the amount of dry matter. The dry matter of the moss grown in the solution containing levulose is much greater than that of the moss grown in the solution containing glucose. In the light twice as much dry matter was formed with levulose as the carbon source than with glucose as the source of carbon. In the dark there was produced in the levulose solution 7 times as much dry matter as was formed in the glucose solution.

TABLE II

Solution	Average dry weight of moss	Original sugar per 50 cc.	Sugar used per 50 cc.	Sugar over dry weight
	gm.	gm.	gm.	
Levulose in light	0.0634	0.7700	0.1100	1.7
Levulose in dark	0.0854	0.7700	0.1650	1.9
Glucose in light	0.0345	0.8970	0.0440	1.3
Glucose in dark	0.0115	0.8970	0.0360	3.1
Check, no organic carbon in light	0.0074	....	....	....
Check, no organic carbon in dark	Inappreciable	....	....	....

Discussion

It is evident that the moss used in these experiments can absorb and utilize organic carbon. The experiments do not demonstrate that the mosses under field conditions, in competition with both the bacteria and the fungi, benefit from the organic compounds in the soil. They do suggest, however, that if suitable organic compounds are present in the soil solution they will be absorbed and used by the moss with advantage.

The results at present seem to bear little on the problems of the products formed in photosynthesis. It is an interesting fact, however, that starch was formed from the maltose and lactose, although no evidence was found that either of these sugars was hydrolyzed. They may have been hydrolyzed within the moss cells, or the products of hydrolysis may have been assimilated as fast as they were formed. In either of these cases evidence of the hydrolysis would have escaped the methods used in looking for it. It should also be noted that the growth in the lactose solution was very slight. An examination of the moss for the enzymes, maltase and lactase, would seem pertinent.

The differences in the growth in the levulose and glucose solutions are of considerable interest. BROWN and MORRIS (1), working with *Tropaeolum majus*, believe that glucose is more quickly used up for respiration and possibly also for tissue forming than is levulose. LINDET (4), working with the yeast and fungi, concluded that glucose is mainly concerned in respiration, while levulose is more particularly concerned in the elaboration of tissue. In the case of *Ceratodon purpureus* the elaboration of tissue is certainly far greater with levulose than with glucose. The data, although not conclusive, also suggest that the elaboration of tissue in the presence of levulose is more economical than in the presence of glucose, as the sugar used per unit of dry matter formed is generally smaller in the levulose than in the glucose solution.

Summary

1. Under the conditions of the experiments reported organic carbon in the form of levulose, glucose, galactose, lactose, cane sugar, and maltose is absorbed and utilized by *Ceratodon purpureus*.

2. Starch is formed in the dark from levulose, glucose, galactose, lactose, cane sugar, and maltose.

3. Mannite, glycerine, and starch cannot be utilized by this moss.

4. The amount of growth with levulose as the source of carbon is 2–7 times greater than that with glucose as the source of carbon.

5. In the presence of levulose the greater amount of growth occurs in the dark. With glucose the greater amount of growth occurs in the light.

6. Light seems to be necessary for the formation of moss plants, even though available carbohydrate is furnished.

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